IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Monto H. KUMAGAI, *et.al.*

Application Serial No. 09/359,300

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Art Unit: 1636

Examiner: Leffers Jr., G.

Attorney's Docket No: 08010137US07

For: **METHOD OF COMPILING A FUNCTIONAL GENE PROFILE BY TRANSFECTING A NUCLEIC ACID SEQUENCE OF A NON-PLANT DONOR INTO A HOST PLANT IN A POSITIVE SENSE ORIENTATION**

DECLARATION PURSUANT TO 37 C.F.R. §1.132

BOX AF
Assistant Commissioner for Patents
Washington D.C. 20231

Sir:

I, Guy R. Della-Cioppa, do hereby declare as follows:

1. I am a co-inventor of the above-identified Patent Application. I received my Ph.D. in Biology from the University of California at Los Angeles in 1982. I am currently a Vice President of Business Development at Large Scale Biology Corporation. My brief Biographical Sketch is attached as Appendix A.

2. I am familiar with the prosecution history of the above-identified Patent Application. I have read the Final Office Action dated August 30, 2001. I believe that Claim 45, as amended, is not vague or indefinite because a functional gene profile is finite. The limit of a functional gene profile is defined by the cumulative length of all the genes in the genome of a donor organism. As defined in the specification, at page 35, a functional gene profile will depend upon the quantity of donor genes capable of causing over-expression or suppression of host genes or of being expressed in the host organism in the absence of a homologous host gene.

3. I am presenting the following working example to illustrate how a functional gene profile has been compiled according to steps (a) (f) of Claim 45. Although the donor

organism in the example is a plant, the concept of compiling a functional gene profile remains the same in the present invention.

(a) Preparing a library of DNA or RNA sequences from a donor organism, and constructing recombinant viral nucleic acids each comprising an unidentified nucleic acid insert obtained from said library.

Arabidopsis thaliana ecotype Columbia (0) seeds were sown and grown. The entire plant, root, leaves and all aerial parts were collected 4 weeks post sowing. High quality total RNA was isolated from the *Arabidopsis thaliana* tissue using a hot borate method. The yield of total RNA from 60 g of tissue was ~ 15 mg.

Then, mRNA was isolated from total RNA using oligo $(dT)_{25}$ DYNABEADS® (Dynal, Inc., Lake Success, NY). Double strand cDNA was synthesized either with NotI-$(dT)_{25}$ primer or on oligo $(dT)_{25}$ DYNABEADS® based on the manufacturer's instruction (Gibco-BRL superscript system). Typically, 5 μg of poly A^+ RNA was annealed and reverse transcribed at 37°C with SUPERSCRIPT II reverse transcriptase (Stratagene, La Jolla, CA). For the non-normalized cDNA library, double stranded cDNAs were ligated to a 500 to 1000-fold molar excess SalI adaptor, digested by restriction enzyme NotI, and size-selected by column fractionation. Those cDNAs were then cloned directionally into the XhoI-NotI sites of the TMV expression vector, 1057 N/P.

The *In vitro* RNA transcriptions were performed by incubating a transcription mixture containing a 6:1 RNA ratio of cap structure:rGTP, Ambion mMessage Machine buffer and enzyme mix (Ambion, Inc., Austin, TX) with 2 μl of cDNA solution in a 96-well plate.

(b) Infecting plant hosts with said recombinant viral nucleic acids.

In order to prepare for plant inoculation, 90 μl of each encapsidated RNA transcript sample and 90 μl of FES transcript inoculation buffer (0.1M glycine, 0.06 M K_2HPO_4, 1% sodium pyrophosphate, 1% diatomaceous earth and 1% silicon carbide) were combined in the wells of a new 96-well plate. The 96 well plate was then placed on ice.

Nicotiana benthamiana plants were removed from the greenhouse and brought into the laboratory 14 days post sowing. The entire 180 μl of RNA transcript sample from (a), was drawn up and pipetted in equal aliquots (approximately 30 μl), onto the first two true leaves of three separate *Nicotiana benthamiana* plants. The mixture was spread across the

leaf surface and the leaves were abraded so as to allow the encapsidated RNA transcript to enter the plant cell structure.

(c) Transiently expressing the unidentified nucleic acid inserts in said infected plant hosts.

After an entire 32 plant flat was inoculated with encapsidated virus, the plants were grown in a greenhouse. Infected and uninfected (control) plants were grown under identical conditions and an automated visual phenotypic analysis is conducted of each plant.

(d) Determining one or more phenotypic or biochemical changes in said plant hosts.

At 13 days post-inoculation, a visual examination was made to identify plants whose phenotype deviates substantially from plants infected with a control. The phenotypically different plants were divided into regions (for example: shoot apical region, infected phloem source leaves, stem) and descriptive terms were applied to each region to document the visual observation. A matrix-style phenotypic database was created using the LIMS software. This approach was rapid, allowing 96 plants to be described in detail as being infected, not infected with a detailed phenotype in ~15 min.

(e) Identifying said recombinant viral nucleic acids that result in said one or more phenotypic or biochemical changes in said plant host.

The phenotypic data including descriptive of various parts of each plant was entered into a matrix-style database created using LIMS software. Once in the database, the phenotypic results were linked to the sequence data and bioinformatic analysis associated with each of the GENEWARE® vector (i.e. each cDNA in the library).

(f) Identifying donor genes or plant host genes associated with said one or more phenotypic or biochemical changes; whereby a functional gene profile of said plant host or said donor organism is compiled.

Out of over 8000 *Nicotiana benthamiana* plants infected, 109 were discovered that exhibited a dwarf phenotype. Sequence analysis of these cDNAs identified the nucleic acid sequences, which compiled the functional gene profile for a dwarf phenotype.

4. Based on the above working example, a functional gene profile can be compiled step by step according to Claim 45. Therefore, it is my opinion that Claim 45 is not vague or indefinite.

5. I declare further that all statements made herein are of my own knowledge, are true, and that all statements made on information and belief are believed to be true by me; and further that these statements were made with the knowledge that the making of willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the applications or any patent issuing thereon.

Respectfully submitted,

Dated: 2/27/02

Guy R. Della-Cioppa, Ph.D.
Vice President, Business Development
Large Scale Biology Corporation

Biographical Sketch – Guy della-Cioppa

Guy della-Cioppa is an executive officer and twelve-year veteran with Large Scale Biology Corporation (Nasdaq: LSBC). He serves as a key member of the senior management team and represents the company at major scientific and investor conferences. He was a principal member of the LSBC working group that developed the initial business plan for the IPO, served on the team that drafted the business section for the prospectus, and helped create the road show presentation materials for the company's IPO in August, 2000.

Prior to serving in Business Development he served as Vice President,Genomics. In that capacity, he managed many projects, including the development and scale-up phase of the three-year collaboration and license agreement with The Dow Chemical Company. He has extensive experience working with corporate partners and university laboratories on collaborative research projects, licensing, technology transfer and regulatory affairs.

His doctoral degree in biology from UCLA was followed by study as an NIH Postdoctoral Fellow at the Worcester Foundation for Experimental Biology and five years in various senior positions at Monsanto Company in St. Louis.

He has 13 issued US patents, numerous US and international patents pending, has published over 60 peer-reviewed scientific papers, meeting reviews,editorial opinions, and invited book chapters. He serves on the editorial board of OMICS: A Journal of Integrative Biology, and is a frequent invited guest speaker and symposium chair at national and international biotechnology meetings and annual scientific conferences. He is a member of the Licensing Executives Society and has recently completed executive education studies in financial analysis at Stanford University's Graduate School of Business.